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Gas Exchange and Yields of *Bt* Resistant Maize (*Zea mays* L.) with European Corn Borer (*Ostrinia nubilalis*, Hübner) Infestation

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ABSTRACT

Seed companies have developed transgenic maize (*Zea mays* L.) hybrids resistant to European corn borer (ECB) [*Ostrinia nubilalis* (Hübner)]. However, the impact of this Bt (*Bacillus thuringiensis*) gene on other plant processes has not been fully researched. In 1997 and 1999, a study at Central Golden Harvest Research in Clinton, IL focused on how the Bt gene affects gas exchange and yields of maize with and without ECB infestation. Bt and non-Bt isogenic pairs were planted with or without nets and/or insecticides to eliminate natural infestation of ECB, and with or without manual infestation of ECB. Rates of photosynthesis and transpiration were similar for Bt and non-Bt plants regardless of ECB infestation. In 1997 and 1999, yields were not significantly different for Bt and non-Bt plants, but in 1999 yields were significantly higher in plants with nets or insecticides compared to no nets or insecticides. In 1997, kernel mass was significantly higher for Bt than non-Bt plants (28.6 and 26.7 g, respectively, for 100 kernels). In 1999, kernel mass was not significantly different between Bt and non-Bt plants; however for one isogenic pair of hybrids, plants with nets and insecticide had significantly larger kernels than those without insecticide. Seed vigor showed no significant differences for germination percentages or root lengths. Thus, Bt genes had no significant effects on gas exchange or yields regardless of ECB infestation, but had a significant effect on kernel mass in 1997.

INTRODUCTION

European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a pest responsible for great damage to maize in the United States Corn Belt. ECB is responsible for annual maize production losses of several millions of dollars in the northern Corn Belt (Barry and Darrah 1991, Lauer and Wedberg 1999, Russnogle 1997).

One method to combat the destructive behavior of this pest is the insertion of a Bt gene from a soil-bacterium (*Bacillus thuringiensis)* into the corn genome. The inserted Bt gene directs the plant to produce a Cry protein. When ingested by ECB after eating stems and leaves, this protein causes the digestive system of ECB to become inactive (Hyde et al.

1999). Many maize hybrids have been engineered to produce actively the Bt protein only in the leaf and stalk tissues but not in the kernels or pollen (Mason et al. 1996). The protein toxin in the leaf and stalk is an effective control for the first and second generation of the borer (Mason et al. 1996, Traore et al. 2000). A benefit of genetic engineering of maize is the inhibition of the development of ECB, but it also allows farmers to decrease the amount of pesticides applied to the field, which is a beneficial environmental practice.

Transition insects such as boring insects may cause physiological problems by decreasing the photosynthetic rate, and therefore slowing the productivity of the plant (Mason et al. 1996). How the Bt gene affects the maize plant through physiological factors such as gas exchange and water movement is not clear. The Bt gene decreases the damage due to infestation of the European corn borer, but whether a cost occurs as decreased yields, inhibited photosynthesis and transpiration, reduced rates of growth and development, or lower seed vigor is unclear. European corn borer feeding on vascular tissues can influence grain weight by interfering with photosynthate assimilation and movement to the grain (Calvin et al. 1988).

The focus of this study is to determine if the Bt construct incurs a physiological cost or loss in yield to the plant expressing that bacterial gene. Differences between Bt hybrids and their non-transgenic counterparts were compared for photosynthesis and transpiration levels, kernel totals, kernel mass, and seed vigor (from germination and root lengths). These parameters were compared for Bt and non-Bt hybrids with several treatments to alter ECB infestation. These treatments consisted of manual infestation with European corn borer (2 times during growing season), and the use of nets covering the corn or the application of an insecticide to prevent natural ECB infestation.

MATERIALS AND METHODS

Three isogenic pairs of *Zea mays* (L.) hybrids were used: H-2493 and H-2493Bt (1997); H-2581 and H-9481Bt (1999); and H-9345 and 999803Bt (1999) with maturation times of 109, 114 and 114 days, respectively. Choices of hybrid pairs in each year were dependent upon the seed availability from seed banks. Each pair contains a transgenic (resistant) and non-transgenic (susceptible) line. The transgenic hybrids contain the *Bacillus thuringiensis* (Bt) gene. Hybrids were planted at Central Golden Harvest Research, Clinton, IL on May 22, 1997 and May 26, 1999. The plots were planted with a Kinze 4 row cone plot planter (Almaco, Nevada, IA). Plots were 4.6 m long and 0.8 m apart. Seeds were placed at a depth of 5 cm with 25 seeds per row and thinned to 20 plants per row at the five-leaf stage. Each whole plot consisted of all the treatments and each plot was a treatment-hybrid combination consisting of a single row for an area of 3.7 m^2. For each treatment there were 5 replications for 1997 and 4 replications for 1999 in the field study. Seed was planted in a Sable soil which is a poorly drained soil in swales and on flats in the uplands (Windhorn 1991). Both years, in mid-April before planting, nitrogen was added to the soil as a 28% liquid solution (by weight), that was a mixture of ammonium nitrate and urea, marketed commercially as URAN. Dual II (pre-plant) and Atrex 4L (post-emergence) herbicides were applied in 1997 at the recommended rates. Dual II (pre-plant) and Spirit (post-emergence) herbicides were applied in 1999 at the recommended rates. In 1999, an insecticide, Lorsban diluted at 300 ml per hectare, was applied for control of rootworm beetles near mid-flowering. Environmental parameters for heat units and precipitation (Figs. 1 and 2) were recorded at the Golden Harvest weather station (GHWS) in 1997 and 1999 except for March 16 - August 31, 1999 when parameters were recorded at Lincoln, IL weather station due to a lightning strike at the Golden Harvest weather station.

The first year (1997) and second year (1999) consisted of a stratified random arrangement of the hybrids. In 1997 the plots were arranged where the hybrids were either infested with European corn borer (ECB) or sprayed with a pesticide to stop the natural infestation. In 1999, the experimental design was changed to incorporate the use of nets covering the plants within the replications. Nets were used as a deterrent for the natural infestation of corn borer in case pesticides had adverse effects. However, the nets developed large holes due to environmental stress from wind and rain, and were unusable, so pesticides were used again starting at the end of July. Hence, the four treatments consisted of: Nets or insecticide/Infest ECB, Nets or insecticide/No ECB, No nets or insecticide/Infest ECB, and No nets or insecticide/No ECB. Experimental plots utilizing infested insects were separated from the control plots with two rows of border plants to decrease the migration of European corn borer into non-infested treatment plots. To control ECB, plots were sprayed with an insecticide, Pounce 3.2EC (1997) or Capture 2EC (1999) (10% solution of each) once a week, beginning early in July, using a hand held sprayer misting above the canopy to decrease corn borer infestation. The plants in the experimental plots where insects were added, were infested with two separate first instar generations of European corn borer during the early whorl stage (late June) and on the shank of the ear (early August) during both years. The European corn borer egg masses were provided by Garst Seed Company Inc. (Slater, IA). A mixture of finely ground corn cob grits (through 40 mesh screen, The Andersons, Maumee, OH) and newly hatched corn borer larvae were added to a plastic bottle. Larvae were dispensed using a handheld spring-loaded bazooka (Country Plastics, Ames, IA) to release one cubic centimeter =1 “shot” of the mixture onto the plant. Each shot averaged 40 to 60 corn borers. Two shots were applied per plant for each infestation period, providing heavy infestation on the maize.

Photosynthesis and transpiration rates were measured approximately every two weeks beginning June 27, 1997 and July 23, 1999, and ending September 3rd in both years. The LCA-4, Analytical Development Company Infrared Gas Analysis Leaf Chamber Analyzer (Houston, TX) was used to measure photosynthesis and transpiration. The sixth plant of every row (1997) and three random plants per row (1999) as determined by a random numbers table were used. Leaves used with the LCA-4 were located at the top node with a fully expanded leaf of the plant during vegetative growth and one node above the ear during the reproductive plant growth. Each measurement took 45 seconds to complete, and measurements began during the peak sunlight period starting at 10:00 AM and ending as late as 5:00 PM. All LCA-4 sessions began with the first replication.

Harvest included the removal of the first 10 ears from each row in 1997, and all ears in 1999. We determined the seed was ready for harvest by first noticing a physiological maturity of the plant and also checking for the blackened abscission layer on the kernel. A change of color for the abscission layer indicates the end of active transport so no dry matter is accumulating in the kernel. Seed was removed from the cob and counted using one of two light beam seed counters, i.e. FMC Corp., Seed Burro 801, Homer City, PA

(1997) and International Marketing and Design Corp., Old Mill #900, San Antonio, TX (1999). Total number of seed was counted for each plot in 1997 and in 1999. Ten (1997) and eight (1999) samples of 100 kernels per row were used in seed weight studies. Kernel mass was determined after drying at a temperature of 40˚C for 10 days.

One mixed sample of kernels per row was used to determine germination percentages (both years) and root lengths (1999). The ragdoll method was used in the greenhouse (1997) and the Percival Scientific Inc. (Boone, IA) seed germinator (1999). In 1997, five ragdolls (rolled paper towels, Envision Acclaim, Fort James, Deerfield, IL) of 20 kernels each from each plot were placed on a plastic plant tray (52 x 25 x 7 cm) and put in moist conditions for seven days. The number of germinated seed was recorded. For the 1999 germination tests, a series of 4 replications was conducted, each lasting for 7 days. Each ragdoll contained 20 kernels with 5 kernels from each hybrid from each of four reps within the same treatment (i.e. nets or insecticide /infest ECB, nets or insecticide/no ECB, etc). Sixteen (4 hybrids x 2 nets or insecticides x 2 infestations) ragdolls were used for each of the four replications. The germinator was set for a 16-hour day at a temperature of 25˚C. Upon completion of the incubation period, germinated seed was counted and root lengths were measured.

For 1997 data, a 2-way ANOVA was used incorporating corn hybrids and infestation of corn borers. The 1999 data were analyzed by 3-way analysis of variance (ANOVA). Sources of variation were corn hybrids, use of nets/insecticides, and the infestation of European corn borer. A separate ANOVA test was used for each isogenic pair. The statistical program, Co-Stat (1986), was used. The Duncan's multiple range test was used to separate means at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Photosynthesis and transpiration rates for 1997 and 1999 showed no significant differences between individuals within an isogenic maize pair any time over the season (Fig. 3). In 1997 the photosynthesis and transpiration rates followed a gradual curve from higher levels during early growth to lower levels as the plants matured. These results were different for 1999, showing an approximate 25 μmol m^{-2} s^{-1} difference for photosynthesis and 6 mol m^{-2} s^{-1} difference for transpiration during late July between years. However, as the plants matured, rates balanced between the two seasons. Environmental parameters may have hindered the photosynthesis and transpiration levels in the beginning months of the 1999 season. As shown in Figs. 1 and 2, high temperatures and low rainfall in late June and most of July might have caused the plants to conserve water therefore decreasing photosynthesizing capabilities.

In 1999, a block effect occurred (results not shown) for both photosynthesis and transpiration rates between the replications, being highest in replication 1 and lowest in replication 4. This effect possibly was due to changes in light levels over the day. All of the photosynthesis and transpiration measurements began at the same time (10:00 AM) during both years. In 1997 only one plant was measured per plot in each treatment over all replications, and measurements were completed by 2:00 PM (i.e. during peak sunlight of the day) which could account for active gas exchange in plants throughout the sampling time. In contrast, in 1999, three plants were measured per treatment in each plot over all replications, which increased time required for data collection. Measurements did not conclude until 5:00 PM (i.e. past the peak sunlight of the day) which could account for lower gas exchange near the end of the sampling time.

Other factors were the use of nets/insecticides to exclude insects or the manual infestation of European corn borer to the plants which neither showed significant differences in gas exchange (data not shown). According to Godfrey et al. (1991) in a 2-year field study, European corn borer larval tunneling in 1987 significantly reduced corn photosynthetic rates by 11.4 and 22.1% with 3 and 5 larvae per plant, respectively, whereas 1 larvae per plant infestation significantly increased photosynthetic rate. These data did not correlate with our data where no significant differences in photosynthesis between infested or non-infested hybrids were seen. Other studies show that with heavy infestation of ECB to the corn plants, higher photosynthetic and lower transpiration rates in the non-transgenic hybrids might occur, considering the plant responses to the insect stress by producing more sugars to compensate for the damage done to xylem and other tissues by boring insects (Calvin et al. 1988).

Seed totals for 1997 hybrids showed no significant difference between the members of an isogenic pair (H-2493 vs. H-2493Bt), or any interaction between the hybrids and the use of insecticides or the infestation of European corn borer (Table 1). In 1999, no significant difference was found between hybrids for seed totals for the members of the isogenic pairs (H-2581 vs. H-9481Bt) or (H-9345 vs. 999803Bt) although plants within treatments using nets/insecticides to exclude European corn borer from the plots had higher seed totals than plants without the nets/insecticides (Table 2).

In 1997, seed mass of Bt and non-Bt isogenic pairs were significantly different with an increased mass for Bt plants (Table 1). No significant difference was found between treatments with insecticides or with ECB infestation in 1997 (Table 2). In 1999, seed mass was higher for plots treated with nets/insecticides than for non-treated plots for the isogenic pair H-2581 and H-9481Bt (Table 2). For the second isogenic pair, H-9345 and 999803Bt, no significant differences occurred between any of the factors.

Differences in seed vigor for Bt isogenic pairs were estimated by utilizing germination percentages and root lengths (Table 3). These data show no significant difference between members of isogenic pairs of hybrids in 1997 and 1999.

Agricultural reports show that Bt hybrids have significantly higher yields when compared to non-Bt plants, when under moderate to severe levels of ECB infestation, in contrast to our results. Current estimates of 5% yield loss per borer per plant for first generation ECB and a 3% yield loss per borer per plant for second generation are used widely (Mason et al. 1996, Steffey and Gray 1994). According to Graeber et al. (1999), non-transgenic hybrids showed a decrease in yield of 6.3% with first and second ECB infestation compared to a treatment without infestation. When infested with both ECB generations, the susceptible (non-transgenic) hybrids yielded significantly less (6.6%) than their Bt counterparts. A three-year study by Pioneer Seed Company shows that Bt hybrids exhibit a 1.6 bushel/hectare yield advantage under low ECB infestation, a 5.2 bushel/hectare advantage under medium infestation, and a 11.6 bushel/hectare yield advantage under high infestation levels compared to hybrids without Bt (Olson 2000).

These data support Bt corn to exhibit higher yields than their non-transgenic counterpart when considering insect infested conditions. Even under heavy infestations in our plots, no significant difference was found between the resistant transgenic and susceptible non-transgenic hybrids. Some reasons for the discrepancies between our data and other studies are possible. One of these reasons could be the size of our plots compared to the larger plot sizes with more replications in the other studies. Smaller plots result in smaller sample sizes, which result in an increased variability. Another reason may be hand harvesting in relation to machine harvesting. Hand harvesting permits all ears to be collected whereas machine harvesting in the other studies may cause broken stalks and detached ears (due to ECB boring) to be missed and seed to be lost. Yet another reason may be the varieties of hybrids used. Our study used only Golden Harvest varieties that express Bt trait in all cells throughout the life of the plant whereas some of the other studies used maize varieties expressing Bt only in green tissue and pollen or in green leaf tissue with active chlorophyll production offering little late season protection.

In summary, apart from the heavier seed with Bt hybrids when compared to its isogenic pair in 1997, no significant differences were found between the isogenic maize hybrids for most comparisons in the study including yields, seed mass or physiological changes whether or not they exhibited the Bt trait. In 1999, we found these plants with the use of nets/insecticides had significantly higher seed totals and kernel mass than those not sprayed. A decrease in physiological activity in 1999 was possibly due to drought stress in the early part of the season (late June to early July). This response may have been a variable during seed production in 1999 affecting seed totals and mass. In conclusion, our results provide some insight into the differences in physiological factors, seed totals, and seed mass with the incorporation of the Bt gene into the corn plant.

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REFERENCES CITED

Barry, D. and L. L. Darrah. 1991. Effect of research on commercial hybrid resistance to European corn borer (Lepidoptera: Pyralidae). Journal of Economic Entomology 84:1053-1059.

Calvin, D. D., M. C. Knapp, K. Xingquan, F. L. Poston and S. M. Welch. 1988. Influence of European corn borer (Lepidoptera: Pyralidae) feeding on various stages of field corn in Kansas. Journal of Economic Entomology 81:1203-1208.

Co-Stat Statistical Analysis. 1986. CoHort Software Company. Version 3.02. Berkley, CA.

Godfrey, L. D., T. O. Holtzer, and J. M. Norman. 1991. Effects of European corn borer (Lepidoptera: Pyralidae) tunneling and drought stress on field corn gas exchange parameters. Journal of Economic Entomology 84:1370-1380.

Graeber, J. V., E. D. Nafziger, and D. W. Mies. 1999. Evaluation of transgenic, Bt-containing corn hybrids. Journal of Production Agriculture 12:659-663.

Hyde, J., M. A. Marshall, P. V. Preckel, and C. R. Edwards. 1999. The economics of Bt corn: valuing protection for the European corn borer. Review of Agricultural Economics 21:442-454.

Lauer, J. and J. Wedberg. 1999. Grain yield of initial Bt corn hybrid introductions to farmers in the northern corn belt. Journal of Production Agriculture. 12:373-376.

Mason, C. E., M. E. Rice, D. D. Calvin, J. W. Van Duyn, W. B. Showers, W. D. Hutchison, J. F. Witkowski, R. A. Higgins, D. W. Onstad and G. P. Dively. 1996. European corn borer-ecology and management. North Central Regional Extension Publ. No. 327. Iowa State University, Ames, IA 57 pgs.

Olson, J. 2000. When does Bt corn pay? Farm Industry News 33:66-72.

Russnogle, J. 1997. Studies show solid Bt payback. Soybean Digest. 24i-j.

Steffey, K. L. and M. E. Gray. 1994. Management of field crop insect pests. University of Illinois Cooperative Extension Service Circ. 1333. Urbana, IL. p. 173-186.

Traore, S. B., R. E. Carlson, C. D. Pilcher, and M.E. Rice. 2000. Bt and non-Bt maize growth and development as affected by temperature and drought stress. Agronomy Journal 92:1027-1035.

Windhorn, Roger D. 1991. Soil survey of De Witt County, Illinois. United States Department of Agriculture, Washington, D.C. p. 74-75.

Table 1. Total number and dry mass of seeds harvested for Bt isogenic pairs of hybrid maize in 1997 and 1999 at Clinton, IL.

Hybrid Pairs	Seed Totals[1] (per treatment)		Seed Mass[2](g)	
	1997			
H-2493	4009	a[3]	26.7	b
H-2493Bt	3867	a	28.6	a
	1999			
H-2581	8087	a	34.9	a
H-9481Bt	7923	a	33.4	a
H-9345	10840	a	27.6	a
999803Bt	9989	a	27.9	a

[1] In 1997, 10 plants were used per treatment to determine seed totals, and in 1999 all plants were harvested in the treatment.
[2] Mass was measured per 100 kernels.
[3] Means followed by a different letter within an isogenic pair in a column are significantly different as determined by Duncan's multiple range test, 5% level.

Table 2. Field use of nets and/or insecticide sprays in the experimental plots for 1997 and 1999 showing seed totals and mass of seed.

Treatment	Seed Totals		Seed Mass[1](g)	
	1997 (H-2493; H-2493Bt)			
Spray (No ECB)	4101	a[2]	27.5	a
No Spray (ECB)	3775	a	27.8	a
	1999 (H-2581; H-9481Bt)			
Nets and Spray (No ECB)	9693	a	35.9	a
No Nets or Spray (ECB)	6316	b	32.4	b
	1999 (H-9345;999803Bt)			
Nets and Spray (No ECB)	11475	a	27.9	a
No Nets or Spray (ECB)	9354	b	27.6	a

[1] Mass was measured per 100 kernels.
[2] Means followed by a different letter within an isogenic pair in a column are significantly different as determined by Duncan's multiple range test, 5% level.

Table 3. Percent germination (1997 or 1999) and root length (1999) for seed from Bt isogenic pairs of hybrid maize after 7 days.

Hybrid Pairs	Germination %	Root Length (cm)
	1997	
H-2493	99 a[1]	N/A
H-2493Bt	97 a	N/A
	1999	
H-2581	98 a	9.2 a
H-9481Bt	95 a	8.4 a
H-9345	96 a	9.1 a
999803Bt	97 a	8.4 a

[1] Means followed by a different letter within an isogenic pair in a column are significantly different as determined by Duncan's multiple range test, 5% level.

Figure 1. Heat units accumulated between April and October from a weather station located 4 kilometers east of Wapella, IL in 1997 and in Lincoln (until August 31) or Wapella, IL in 1999. Heat units are figured between 50 and 86°F.

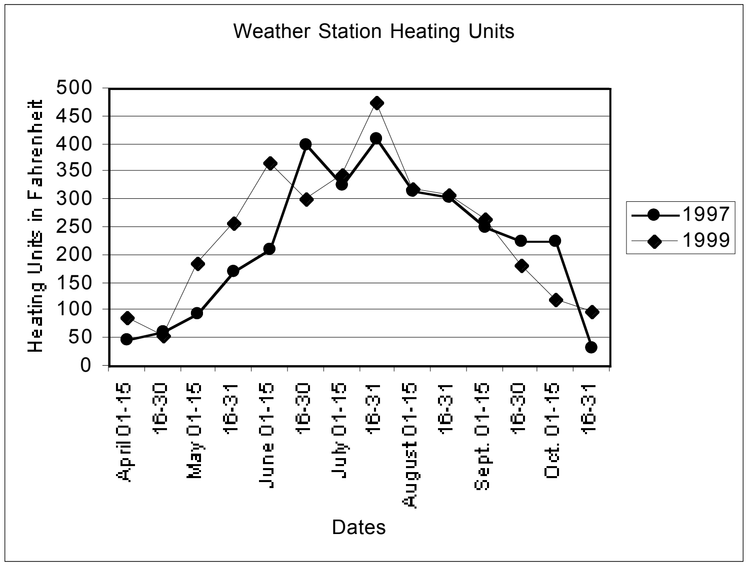

Figure 2. Precipitation between April and October from a weather station located 4 kilometers east of Wapella, IL in 1997 and in Lincoln (until August 31) or Wapella, IL in 1999.

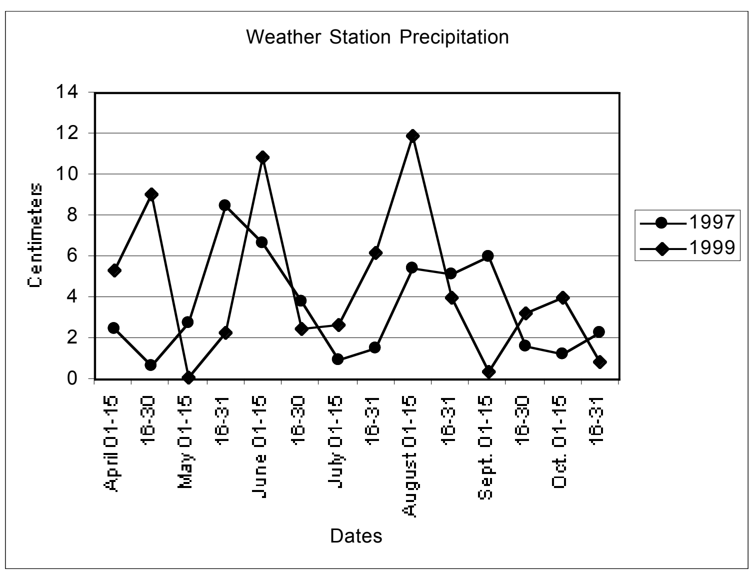

Figure 3. Photosynthesis and transpiration rates of isogenic pairs of corn hybrids over the season in 1997 and 1999 at Clinton, IL. No significant differences between associated isogenic pairs. Three isogenic pairs of hybrids are shown: (H2493, H2493Bt); (H2581, H9481Bt); (H9345, 999803Bt). Data lumped for all nets/insecticides and for all ECB infestations due to no significant interactions.